STIMULATING AND RECORDING DEVICE

Field of the Invention

The present invention relates to devices of the type mentioned in the preambles of the independent claims for experiments on biological tissues or organs.

Prior Art

In order to record the effect of electric stimuli on tissue, for example action potential analysis of cardiac muscle fibres preparations (e.g. Purkinje fibre), a time-consuming and labour-intensive experimental procedure is used. This typically involves (i) careful microdissection, (ii) tie-binding each end of the fibre into a perfusion bath (e.g. using thin nylon threads), (iii) beginning perfusion, (iv) pulling sharp glass electrodes, (v) electrolyte buffer-filling the electrodes, (vi) inserting an electrode into one end of the tied fibre to hopefully access a continuity with the intracellular compartment for stimulation), (vii) inserting an electrode into the other end of the tied fibre to hopefully access a continuity with the intracellular compartment for recording, (viii) filling a perfusion chamber with a buffer and/or drug, (ix) starting a stimulus-record protocol, delivered via one electrode and recorded through the other.

Such a preparation by a skilled operator takes approximately 20 minutes.

Summary of the Invention

The present invention relates to an electrical stimulus-record device which through clamping onto pieces of excitable tissue or organs, provides a simple access method for measuring complex effects of drugs on different receptor and ion channel populations in a biological sample.

According to the present invention, at least some of the problems with the prior art are solved by means of an electrical stimulus-record device having the features present in the characterising part of claim 1.

Brief Description of the Figures

Figure 1 shows a schematic view from above of one embodiment of a device in accordance with the present invention,

Figures 2a)-2c) show enlarged schematic plan, front respectively lateral views of the device from figure 1.

Detailed Description of Embodiments Illustrating the Invention

In the following, any references to orientations such as up, down, upper side, lower side or the like, are intended to be non-limiting and are only used to make it easier to understand the present invention and, unless otherwise stated, they refer to possible embodiments of devices in accordance with the present invention when positioned in the working configuration. Furthermore the skilled person will understand that the relative positions of the components can be reversed and/or rearranged while still remaining in the scope of the present claims.

A first embodiment of a tissue clamping and testing device 1 in accordance with the present invention is shown schematically in fig. 1. Device 1 is shown enlarged in figures 2a)-2c) which are, respectively, plan, front and lateral schematic views of device 1. Device 1 comprises a gripping mechanism formed of a base plate 3 onto which a movable clamping frame 5 is pivotably mounted by means of a hinge 7. Frame 5 comprises two parallel arms 9, each D mm (e.g. 10 mm) long, w mm wide (e.g. 1 mm) and t mm (e.g. 1mm thick), spaced a distance d (e.g. 2 mm) apart and each attached by a first end 11 to hinge 7. Frame 5 is pivotable from a working position, in which it is substantially parallel to the surface of base plate 3 with the arms 9 in a tissue clamping position over a tissue placement region 13 (and in which working position it is intended that in use it would be in clamping contact with the tissue being sampled), to a non-working position where arms 9 are not in a tissue clamping position over tissue placement region 13 (and in which non-working position it is intended that in use it would not be in clamping contact with the tissue being sampled) . The second end 15 of each arm 9 is joined to a buffer chamber 17 mounted on, and extending between, arms 9. Buffer chamber 17 comprises a tubular open-ended bath portion 19 attached to the under surface of the periphery of a central hole 21 formed in a flat plate 23 which is mounted on the upper surface of second end of arms 9. Bath portion 19 protrudes a distance p mm (e.g. 1 mm) which is sufficient to ensure that when it is clamped into contact with a piece of tissue

its periphery forms a seal against the piece of tissue. A recording electrode 25 is positionable in bath portion 19. Recording electrode 25 is sufficiently long enough to penetrate sufficiently deep enough into the tissue so as to ensure electrical contact with living cells inside the tissue and is connectable to a recording means such as a computer (not shown). A bath electrode 26 positioned inside buffer chamber 17 and connectable to the recording means completes the recording circuit with the recording electrode. Buffer chamber 17 may contain a buffer liquid 16 which prevents the tissue from drying out and the buffer liquid may be supplied with, or exchanged for, other liquids such as nutrient liquids or drugs or drug candidates or the like. The same or a different liquid 18 may be provided in the space between the buffer chamber 17 and the frame arms 9.

The underside 27 of each arm 9 is provided with a plurality of electrode teeth 29 resp. 29' which project down out of underside 27. Preferably electrode teeth 29, 29' are made of insulating material and have conducting tips that are sufficiently long enough to penetrate sufficiently deep enough into the tissue so as to ensure electrical contact with responsive cells inside the tissue. Preferably the teeth penetrate to a depth of from 0.05 mm to 2.0 mm, more preferably from 0.2 mm to 1.0 mm. The portion 33 of the base plate under the buffer chamber 17 and extending beyond the electrode teeth 29, 29' is intended to receive a piece of tissue 35 and this tissue receiving portion 33 may be provided with a gasket (not shown) in order to keep the tissue moist. The electrode teeth 29, 29' are pointed in order to be able to penetrate the piece of tissue 35 and provide good electrical contact with the piece of tissue 35. Electrode teeth 29, 29' are connectable to a power supply (not shown) which can be controlled, for example by a computer, to provide stimulating pulses to the piece of tissue.

Frame 3 is preferably biased into the working position e.g. by biasing means such as a spring (not shown), and/or is provided with locking means (not shown) for releasably locking it in the working position.

A method of using device 1 to hold a piece of tissue comprises the following steps:

1) frame 3 is moved to a non-working position;

2) a piece of tissue 35 is placed on tissue receiving portion 33;

3) frame 3 is moved to the working position so that electrode teeth 29, 29' and recording electrode 25 penetrate into piece of tissue 35, thereby simultaneously clamping the tissue into place and establishing electrical contact with it.

A method of experimenting on a piece of tissue may comprise the further steps of:
4) applying stimulation pulses through electrodes 29, 29':
5) recording through recording electrode 25 and bath electrode 26 the electrical signal produced by the tissue as a response to the stimulation pulses;
6) changing the composition of the fluid in buffer chamber 17 and recording the electrical signal produced by the tissue as a response to the stimulation pulses and the change of the composition of the buffer fluid.

Thus device 1 is able to produce a rapidly-actuating, but strong and reliable physical clamp of the tissue being tested which serves to overcome the confounding effects of excitation-induced contraction. The frame 3 also enables good electrical contact for delivery of stimuli at the periphery of the tissue 35, and at the same time the central region of the tissue is automatically punctured by the recording electrode 25.

Figures 3a) and 3b) show a second embodiment of the present invention in which a simple gripping mechanism is provided with many recording electrodes that can be reliably and easily inserted at the same time by a device 41 provided with an array of recording electrodes 55. The device is further provided with drug introducing means to introduce drug-containing solutions either to a surface of the tissue 35. Array 43 comprises a quadratic planar substrate 45 with one face 47 intended to be brought into contact with a piece of tissue 35. Face 47 may be provided with a central quadratic grid 49 of microgrooves 51 which surround a plurality of electrode islands 53. Each electrode island 53 is in the shape of a square protuberance and is provided with a central recording electrode 55 which projects a short distance h (e.g. 0.05 - 1.0 mm) out of the face 47 and which, when in use, is intended to penetrate a piece of tissue sufficiently deep enough to establish a reliable electrical contact with it. The electrodes 55 extend though substrate 45, project out of the second face 57 of substrate and are connected to a data buss (not shown). Each electrode is surrounded by an insulating electrode sheath 59 which during use seals against the piece of tissue and prevents short-circuit currents between electrodes. Two opposite edges of substrate 45 are provided with longitudinal clamping

electrodes 61 which project a short distance k (e.g. 0.05 – 2.0 mm, preferably 0.1 –1.0 mm) out of the face 47 and which, when in use, are intended to penetrate a piece of tissue sufficiently deep enough so as to establish electrical contact with the tissue and to resistant contractile forces from the tissue. Microgrooves 51 may be used to allow drugs and other fluids to be introduced and to flow across the substrate 45 and therefore are made deep enough to avoid then being filled with tissue when the substrate 45 is clamped against a piece of tissue. Drugs and fluids may be introduced to the microgrooves via an inlet hole 63 which extends through substrate 45 at one corner of the grid 49, and they may be removed from the microgrooves via an outlet hole 65 which extends through substrate 45 at the opposite corner of the grid 49. A clamping frame 71 (shown schematically by dashed lines) is able to be position over the face 47 of the substrate and can be moved towards the face 47 in order to force the electrodes to penetrate tissue 35.

A device in accordance with the present invention enables continuous delivery of electrical stimuli and recording of responses via electrodes while dipping the device 1, 41 into different drug-containing or wash solutions, a procedure that can be carried out by an operator or robot arm or laboratory transport system. Preferably the ends of the electrodes are made sharp enough to penetrate solid tissue (e.g. with pointed (conical) tips).

Embodiments of the present invention may use solid-state stimulus-recording electrode pairs. In such embodiments the arrangement and spacing of the stimulus electrode(s) and recording electrode(s) together with the appropriate insulating barriers are defined to maximise the number of simultaneous output signals (or facilitate their sequential recording) while minimising cross-talk between adjacent electrode pairs. Each electrode pair in an array may be programmable for sequential stimulus and recording, as commonly practised in the field, on sample tissues sliced and placed in a physical clamp, which is an intrinsic part of the electrode device. In arrays, the individual stimulus-record (S-R) electrode pairs may be packed closely together but separated by insulation to prevent cross-talk.

In devices in accordance with the present invention as shown in figures 3a) and 3b), the tissue sample is preferably kept moist, and its waste products removed, to increase its life span and is preferably locally perfusable/saturatable with drugs that can permeate to the cells being recorded.

In devices in accordance with the present invention it is preferable that the penetration and holding frame should be of sufficient tenacity to overcome potential problems with elicited tissue contraction. Monitoring of these physical forces may be achieved by incorporation of a strain transducer between different points on the electrode array.

It is conceivable to use devices in accordance with the present invention to perform experiments on a complete organ, for example a mouse, rat or rabbit heart. Such devices for performing experiments on such organs are preferably provided with means for keeping the organ alive during the experiment as well as means for perfusing the organ with, preferably oxygenated, drug-containing solutions.

Devices in accordance with the present invention which clamp on to a tissue or organ sample while rendering it stimulatable and recordable, bring improvements (viz. rapidity, quality of device-to-tissue seal/access, consistency of recording, simplicity) over traditional approaches and enable presentation of the 'captured' tissue or organ to drug-containing and wash solutions in hitherto difficult formats (e.g. the tissue or organ and the stimulus-record device as a unit may be inserted on to larger arrays or data buss devices for batch processing/ recording and/or dipped in solutions controlled by a robot).

The above mentioned embodiments are intended to illustrate the present invention and are not intended to limit the scope of protection claimed by the following claims.